

Payment of fees and expenses

European Patent Office Treasury and Accounts 80298 München Germany Fax +49(0)89 2399-4465

Please con	plete in typescript only	Payará reference	
	na Dzieglewska	77.68.85733	
Fra	nk B. Dehn & Co.	Made of payment Name of bank where EPO account held	
Address			
St. E	ride's House, 10 Salisbury Square		
Lone	on EC4Y 8JD	Deposit account No.	
02 Eng	and	Seponary with the EPO is requested.	
Γ	Patent application/patent No. (please	use a separate form for each application)	
03 EP	03745226.5	PCT	
<u> </u>	Code	Currency Amount	
04	001 Filing fee - EP direct	EUR	
05	002 Search fee	EUR	
06	005 Designation fee(s) ¹	EUR	
07	015 Claims fee(s) (Rules 45(1), 162(1) EPC)	EUR	
08	055 Additional copy	EUR	
09	006 Examination fee	EUR	
10	007 Fee for grant including fee for printing (up to 35 pages)	EUR	
11	OO8 Additional fee for printing (more than 35 pages)	EUR	
12	033 Renewal fee for the 3rd year	EUR	
13	034 Renewal fee for the 4th year	EUR	
14	035 Renewal fee for the 5th year	EUR	
15	020 Filling fee – entry EP phase	EUR	
16	Extension fee(s) for 4:	EUR	
17	122 Fee for further processing	EUR 210.00	
18		EUR	
19		EUR	
20		EUR	
21		EUR	
22		Total EUR 210.00	
Signatu	Monaro Dellino	London, 12 September 2008	

na and SeningsVkizinglevskavLocal Senings/Tamporwy Interior FilenCULKIVED) etalans 12 9 2001 etans (2).doo-12/09/2008

- 59 -

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

- A method of modulating sphingosine kinase functional activity in vitro, said 1. method comprising contacting said sphingosine kinase with an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of said sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.
- A method of modulating cellular activity in vitro, said method comprising 2. contacting said cell with an effective amount of an agent for a time and under conditions sufficient to modulate the phosphorylation of sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.
- The method according to claim 1 or 2 wherein said sphingosine kinase is human 3. sphingosine kinase.
- The method according to any one of claims 1-3 wherein said phosphorylation is 4. modulated at S²²⁵.
- · The method according to claim 4 wherein said agent binds, links or otherwise associates with S225.
- The method according to any one of claims 1-5 wherein modulation of said 6. phosphorylation is modulation of proline-directed protein kinase catalysed phosphorylation.
- The method according to claim 6 wherein said proline directed kinase is ERK1, 7. ERK2 or CDK2.

- 60 -

- 8. The method according to claim 7 wherein said proline directed kinase is ERK2.
- 9. The method according to any one of claims 1-8 wherein said modulation is down-regulation.
- 10. An agent which antagonises the interaction between sphingosine kinase and a phosphorylation catalyst for use in therapeutically downregulating inflammation or cellular proliferation.
- 11. An agent which agonises the interaction between sphingosine kinase and a phosphorylation catalyst or which acts as a phosphorylation catalyst for use in therapeutically stimulating cellular proliferation or inflammation.
- 12. The agent according to claim 10, wherein said agent is for use in the treatment of a condition which is characterised by inflammation or unwanted cellular proliferation in a mammal.
- 13. The agent according to any one of claims 10 to 12 wherein said sphingosine kinase is human sphingosine kinase.
- 14. The agent according to any one of claims 10-13 wherein said phosphorylation is modulated at S²²⁵.
- 15. The agent according to claim 14 wherein said agent binds, links or otherwise associates with S²²⁵.
- 16. The agent according to any one of claims 10-15 wherein said phosphorylation catalyst is a proline-directed protein kinase.
- 17. The agent according to claim 16 wherein said proline directed protein kinase is ERK1, ERK2 or CDK2.

14 December 2 and September September Local September Temporary Interior Floridal KIVBD claims 12 9 2001 claim (2),406-12.09/200

- 61 -

- 18. The agent according to claim 17 wherein said proline directed kinase is ERK2.
- 19. The agent according to claims 10-11 or 12-18 wherein said inflammation is induced by TNF.
- 20. The agent according to claim 10 or 12-18 wherein said cellular proliferation is neoplastic proliferation, TNF-induced cellular proliferation and/or anti-apoptotic activity.
- 21. The agent according to claim 10 or 12-18 wherein said inflammation is inflammatory mediator production and/or adhesion molecule expression.
- 22. The agent according to claim 10 or 12-18 wherein said inflammation is associated with rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.
- 23. Use of an agent in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by inflammation or unwanted cellular proliferation, wherein said agent antagonises the interaction between sphingosine kinase and a phosphorylation catalyst.
- 24. Use according to claim 23 wherein said sphingosine kinase is human sphingosine kinase.
- 25. Use according to any one of claims 23-24 wherein said phosphorylation is modulated at S²²⁵.
- 26. Use according to claim 25 wherein said agent binds, links or otherwise associates with S²²⁵.
- 27. Use according to any one of claims 23-26 wherein said phosphorylation catalyst is

Document and Semigri Miniciples stational Semigrifum person Property Control Control 1273 000 0000 (1

- 62 -

a proline-directed protein kinase.

- 28. Use according to claim 27 wherein said proline directed kinase is ERK1, ERK2 or CDK2.
- Use according to claim 28 wherein said proline directed kinase is ERK2.
- Use according to claim 23-29 wherein said inflammation is induced by TNF.
- 31. Use according to claim 23-29 wherein said condition is a neoplastic condition.
- 32. Use according to claim 23-30 wherein said inflammation is inflammatory mediator production and/or adhesion molecular expression.
- 33. Use according to claim 23-30 or 32 wherein said inflammatory condition is rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.
- 34. An isolated sphingosine kinase variant comprising a mutation at one or more of S¹⁴⁸, S¹⁸¹, Y¹⁶⁴, S²²⁵ or T²⁵⁰, wherein said variant exhibits ablated or reduced phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.
- 35. An isolated sphingosine kinase variant comprising a mutation at one or more of S¹⁴⁸, S¹⁸¹, Y¹⁸⁴, S²²⁵ or T²⁵⁰, wherein said variant exhibits enhanced or up-regulated phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.
- 36. The isolated variant of claim 34 wherein said variant comprises an amino acid sequence with a single or multiple amino acid substitution and/or deletion of amino acid S²²⁵.

- 63 -

The isolated variant of claim 36 wherein said substitution is a Ser²²⁵ Ala 37. substitution.

- 59 **-**

Deleted: G:\Scodata\trigby\c-

Deleted: P:\OPER\TDO\125043 80 claima.doc

Deleted: 12/09/2008 Deleted: 11/09/2008

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of modulating sphingosine kinase functional activity *in vitro*, said method comprising contacting said sphingosine kinase with an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of said sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

Deleted: inducing or otherwise agonlaing and phrasphorylation up-regulates said splringosine kinnes extivity and inhibiting or otherwise orangonising said phosphorylation down-regulates sphingosine kinase activity

2. A method of modulating cellular activity in vitro, said method comprising contacting said cell with an effective amount of an agent for a time and under conditions sufficient to modulate the phosphorylation of sphingosine kinase wherein said agent agents or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

Deleted: inducing or otherwise agonising said phosphorylation up-regulates said cellular activity and labibiting or otherwise untagonising said phosphorylation down-regulates said cellular servity.

- The method according to claim 1 or 2 wherein said sphingosine kinase is human sphingosine kinase.
- 4. The method according to any one of claims 1-3 wherein said phosphorylation is modulated at S²²⁵.
- 5. The method according to claim 4 wherein said agent binds, links or otherwise associates with S²²⁵.
- 6. The method according to any one of claims 1-5 wherein modulation of said phosphorylation is modulation of proline-directed protein kinase catalysed phosphorylation.
- 7. The method according to claim 6 wherein said proline directed kinase is ERK1, ERK2 or CDK2.

8.

- 60 -

The method according to claim 7 wherein said proline directed kinase is ERK2.

Deleted: G:\Secdata\brigby\case\85733\FBD claims 12 9 2008.doc

Deleted: P:\OPER\TDO\125043 90 chims.do

Defeted: 12/09/2008 Deleted: 11/09/2008

- The method according to any one of claims 1-8 wherein said modulation is down-9.
- regulation.
- An agent which antagonises the interaction between sphingosine kinase and a phosphorylation catalyst for use in therapeutically downregulating inflammation or cellular proliferation.
- An agent which agonises the interaction between sphingosine kinase and a phosphorylation catalyst or which acts as a phosphorylation catalyst of sphingosine kinase for use in therapeutically stimulating cellular proliferation or inflammation.
- The agent according to claim 10, wherein said agent is for use in the treatment of a condition which is characterised by inflammation or unwanted cellular proliferation in a mammal.
- 13. The agent according to any one of claims 10 to 12 wherein said sphingosine kinase is human sphingosine kinase.
- 14. The arent according to any one of claims 10-13 wherein said phosphorylation is modulated at S²²⁵.
- 15. The agent according to claim 14 wherein said agent binds, links or otherwise associates with S²²⁵.
- 16. The agent according to any one of claims 10-15 wherein said phosphorylation catalyst is a proline-directed protein kinase.
- 17. The agent according to claim 16 wherein said proline directed protein kinase is ERK1, ERK2 or CDK2.

Deleted: 10. The method according to claim 9 wherein said agent is U0126. ¶

11. The method according to claim 9 wherein said agent is PD98059. ¶

12. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is in a manned, which constant is characterised by shorant, unwanted or otherwise inappropriate cellular activity, said method comprising administering to said manned an offective amount of an agent for a time and under conditions sufficient to modulate sufficient to modulate
phosphoryhtion of sphingosine
kinase whereth inducing or
otherwise agomising said
phosphoryktion up- regulates sald
cellular settivity and inhibiting or
otherwise antagonising said
otherwise shape despect of large phosphorylation down-regulares said cellular activity. ¶ ... [

Deleted: 14

Deleted: method

Deleted: 12 or 13

Deleted: 15

Deleted: method

Deleted:

Deleted: 12-14 Deleted: 16

Deleteds method

Deleted: 15

Deleted: 17

Deleted: method

Deleted: 12-16

Deleted: medulation of said phosphorylation

Deleted: modulation of

Deleted: criniysed

phosphory larior Deleted: 18

Deleted: method

Deleted: 17

Deleted: G:\Secdata\brigby\c texts/85733/FBD claims 17 ... [2] Deleted: P:\OPER\TDO\

Deleted: 12/09/2008 Deleted: 11/09/2008 - 61 -Deleted: 19 18. The agent according to claim 17 wherein said proline directed kinase is ERK2. Deleted: method Deleted: 20. The metho 19. The agent according to claims 10-11 or 12-18 wherein said inflammation is induced by TNF. Delebed: 20 Deleted: collular activity Deleted: 22 20. The agent according to claim 10 or 12-18 wherein said cellular proliferation is Deleted: method peoplastic proliferation, TNF-induced cellular proliferation and/or anti-apoptotic activity. Delebed: 21 Detebed: condition Deleted: a 21. The agent according to claim 10 or 12-18 wherein said inflammation is Deleted: condition and s ... [5] inflammatory mediator production and/or adhesion molecule expression. Deleted: characteristic Deleted: 23. The metho Delebed: method 22. The agent according to claim 10 or 12-18 wherein said inflammation is associated Deleted: with rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory Deleted: 23 Deleted: U bowel disease. Deleted: molecular Deleted: 25 23. Use of an agent in the manufacture of a medicament for the treatment of a Delebad: method Deleted: 23 or 24 condition in a mammal, which condition is characterised by inflammation or unwanted Deleted: inflammatory cellular proliferation, wherein said agent antagonises the interaction between sphingosine Deleted: 26. The method Delebed: aberrant kinase and a phosphorylation catalyst. Deleted: or otherwise Deleted: activity 24. Use according to claim 23 wherein said sphingosine kinase is human sphingosine Deleted: modulares th ...[10] Deleted: 29. Use of a ... [11] kinasc. Deleted: 28 or 29 Deleted: 31 25. Use according to any one of claims 23-24 wherein said phosphorylation is Deleted: 28-30 modulated at S²²⁵. Deteted: 32 26. Use according to claim 25 wherein said agent binds, links or otherwise associates Delebed: 31 with S²²⁵. Deleted: 33 27. Use according to any one of claims 23-26 wherein said phosphorylation catalyst is Deleted: 28-32 Deleted: medulation of

FRANK B.DEHN

2 Victorian Part Column (Middler Resolution State Column Transact France Column 12 5 500 History 12 00 5000 2	Deleted: G:\Scodam\brighy\c- texts\85733\FBD ctaims 12 9 2008.doc
	Deleted: P:\OPER\TDO\125043 80 chims.doc
- 62 -	Deleted: 12/09/2008
- 02 -	Deleted: 11/09/2008
	Deletted: modulation of
a proline-directed protein kinase.	Deletizad: catalysed phosphorylation
	Deleted: 34
28. Use according to claim 27 wherein said proline directed kinase is ERK1, ERK2 or	Deleted: 33
CDK2.	
	Deleted: 35
29. Use according to claim 28 wherein said proline directed kinase is ERK2.	Deleted: 34
	Deleted: 36. Use according to
30. Use according to claim 23-29 wherein said inflammation is induced by TNF.	any one of claims 28-35 wherein said modulation is down [12]
30. Use according to claim 23-29 wheatom one game	Deleted: 36
and the secondition	Deleted: cellular activity
31. Use according to claim 23-29 wherein said condition is a neoplastic condition,	Deleted: 38
<u> </u>	Deleted: 37
32. Use according to claim 23-30 wherein said inflammation is inflammatory mediator	Deleted: and said coll[13]
32. Use according to claim 23-30 wherein said initiation was a	Deleted: 39. Use acco [14]
production and/or adhesion molecular expression.	Deleted: 39
	Deleted: ia
and the standard inflammatory condition is	Deleted; 41
33. Use according to claim 23-30 or 32 wherein said inflammatory condition is	Delebad: 39 or 40
rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel	
discase.	
	Deleted: 42. Use pure [15]
34. An isolated sphingosine kinase variant comprising a mutation at one or more of	Deleted: 1
34. An isolated spiningosine kinaso (managed at the spining of the	Deleted: 7[16]
S ¹⁴⁸ S ¹⁸¹ V ¹⁸⁴ S ²²⁵ or T ³⁵⁰ , wherein said variant exhibits ablated or reduced	Deleted; in a region of [17]
phosphorylation capacity relative to wild-type sphingosine kinase or a functional	Formatted: Superscript
phosphoryamen are allowed thereof	Formatted: Superscript
derivative, homologue or analogue thereof.	Formatted: Superscript
	Formatbed: Superscript
35. An isolated sphingosine kinase variant comprising a mutation at one or more of	Formatted: Superscript
S ¹⁴⁸ S ¹⁸¹ Y ¹⁸⁴ S ²²⁵ or T ²⁵⁰ wherein said variant exhibits enhanced or up-regulated	Deleted: 47
Sies Glot Vist So or I wherein said variant with the sies or a functional	Deleted: in a region of [18]
phosphorylation capacity relative to wild-type sphingosine kinase or a functional	Formatted: Superscript
derivative, homologue or analogue thereof.	Formatted: Superscript
unition (1)	Formatted: Superscript
distribution and accompanies on amino acid	Formatted: Superscript
36. The isolated variant of claim 34 wherein said variant comprises an amino acid	Formatted: Superscript
sequence with a single or multiple amino acid substitution and/or deletion of amino acid	Delebed: 48
	Deleted: 46
S ²²⁵ .	

- 63 -

37. The isolated variant of claim 36 wherein said substitution is a Ser²²⁵ Ala substitution.

Deleted: 49

- The method according to claim 9 wherein said agent is PD98059.
- 12. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate cellular activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of sphingosine kinase wherein inducing or otherwise agonising said phosphorylation up- regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity.
- 13. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate sphingosine kinase functional activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of sphingosine kinase wherein inducing or otherwise agonising said phosphorylation up-regulates said sphingosine kinase functional activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said sphingosine kinase functional activity.

Hanna Dziegłewska 12/09/2008 17/18/00

Page 59 [3] Deleted Transport Hanna Dziegłewska 12/09/2008 17/18/00

Page 51: [4] Deleted Transport Hanna Dziegłewska 12/09/2008 10:58/00

Page 51: [4] Deleted Transport Hanna Dziegłewska 12/09/2008 10:58/00

20. The method according to any one of claims 12-19 wherein said modulation is down-regulation.

21

23. The method according to claim 21 wherein said condition is an inflammatory condition and said cellular activity is the production of inflammatory mediators.

24

Page 61:[7] Deleted 48:00 inflammatory condition is

Page 61: [8] Deleted

11/09/2008 10:59:00

26. The method according to any one of claims 20-25 wherein said agent is U0126.

The method according to any one of claims 20-25 wherein said agent is 27. PD98059.

28

11/09/2008 11:00:00 Page 61: [9] Deleted or otherwise inappropriate

Page-61:[40] Defetted. 11:00:00 modulates the phosphorylation of sphingosine kinase and wherein inducing or otherwise agonising said phosphorylation up-regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity

Page 61: [11] Deleted 11:00:00 Use of an agent in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate sphingosine kinase activity, wherein said agent modulates the phosphorylation of sphingosine kinase and wherein inducing or otherwise agonising said phosphorylation up-regulates said sphingosine kinase activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said sphingosine kinase

30

activity.

Page 62::[12] Deleted 11:02:00 Use according to any one of claims 28-35 wherein said modulation is downregulation.

37

and said cellular activity is TNF-induced cellular proliferation and/or anti-apoptotic characteristic

11/09/2008 11:02:00

Page 62: [14] Deleted -Use according to claim 37 wherein said condition is an inflammatory condition and said cellular activity is the production of inflammatory mediators.

40

Use according to any one of claims 36-41 wherein said agent is U0126.

- Use according to any one of claims 36-41 wherein said agent is PD98059. 43.
- A pharmaceutical composition comprising an agent, which agent modulates 44. phosphorylation of sphingosine kinase, together with one or more pharmaceutically acceptable carriers and/or diluents when used in accordance with the method of any one of claims 1-27.
- An agent, which agent modulates phosphorylation of sphingosine kinase, 45. when used in accordance with the method of any one of claims 1-27.

46

Page 62: [17] Deleted 11/09/2008;11:03:00 in a region of said sphingosine kinase which region comprising a phosphorylation

Page 62: [18] Deleted: 11:04:00 in a region of said sphingosine kinase which region comprising a phosphorylation site